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Long-term symptomless HIV-1 infection in recipients of blood products from a single donor

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There have been reported cases of long-term symptomless human immunodeficiency virus type 1 (HIV-1) infection, but it is not clear whether the benign course of infection was due to host, viral, or other unknown factors. During follow-up of subjects with transfusion-acquired HIV-1 infection in New South Wales, Australia, we identified a group of 6 subjects who had been infected through a single common donor. We were therefore able to study the contributions of various factors to the course of infection.

Throughout follow-up (range 6·8–10·1 years after infection), 5 of the recipients and the donor (last follow-up 10·2 years after infection of the first recipient) remained clinically free of symptoms, with normal CD4 cell counts and no p24 antigenaemia. HIV-1 was isolated from only 1 recipient; the isolate did not induce syncytia in a SUPT1 co-culture assay and had a limited in-vitro host range. 1 infected recipient (who had received extensive immunosuppressive treatment for systemic lupus erythematosus) developed *Pneumocystis carinii* pneumonia and died 4·3 years after infection. The frequency of progression to AIDS or a CD4 cell count below $0·50 \times 10^9$/l was significantly lower among the 6 subjects with a common donor (1/6) than among 101 other HIV-infected transfusion recipients for whom data from 7 years of follow-up were available (94/101; $p < 0·0001$).

These findings suggest that the subjects were infected by a less virulent strain of HIV-1. The identification of this group of subjects should stimulate a search for other similar groups, which will provide important information on the immunopathogenesis of HIV-1 disease.

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Introduction

After primary infection with human immunodeficiency virus type 1 (HIV-1) the risk of severe immunodeficiency and symptoms of disease increases with time.[1-6] The median time to development of acquired immunodeficiency syndrome (AIDS) in HIV-1-infected people is 7–10 years.[1,2,7] However, it is still not clear what proportion of those infected will eventually develop severe HIV-1 disease.

There have been a few reports of people who have been infected for long periods but who have remained symptom-free with a normal absolute number of peripheral CD4 cells.[2,8] It is not clear whether the benign course is due to

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host factors, viral factors, or other unknown factors. We describe here a group of 6 transfusion recipients who were infected by blood or blood products from a single donor between 1982 and 1984, but who have remained well. Thus, we were able to study the contributions to outcome made by host and viral factors.

Subjects and methods

We established a registry of people known to have acquired HIV infection through transfusion of blood in New South Wales; it did not include recipients who also had haemophilia. Cases were identified through the tracing of blood units from donors known to have HIV infection, or through testing for HIV infection either because disease symptoms were being investigated or because the subject was worried that a previous blood transfusion might have led to HIV infection. Every case reported to the registry was followed up until June 30, 1992, for the development of an AIDS-defining illness or death. CD4 cell counts were done regularly.

Among the 118 cases, we identified 6 subjects, all white, who were infected through units of blood or blood products from a common donor over a 2-year period. In 5 of these 6 recipients, and in the donor, long-term follow-up had shown persistent symptomless infection and normal laboratory results. Regular follow-up included history, physical examination, full blood count, T-cell subset counting, and measurement of serum p24 antigen and β_2-microglobulin concentrations. Samples of serum and peripheral-blood mononuclear cells (PBMC) from these subjects were stored at −70°C. The 6th subject died shortly after HIV-1 infection was diagnosed.

The absolute number of circulating lymphocytes was counted on a Coulter counter (Model S Plus IV; Coulter, Hialeah, Florida, USA). Counting of T-cell subsets was done on cells obtained by the whole blood lysis method (Q-Prep; Coulter). The percentages of CD4 and CD8 lymphocytes were determined by direct immunofluorescence with monoclonal antibodies (Ortho Diagnostics, Raritan, New Jersey, USA, and Coulter) against CD3, CD4, and CD8. The samples were analysed on an Epics V flow cytometer (Coulter).

Serum samples were tested for HIV-1 antibodies by an enzyme-linked immunosorbent assay (Genetic Systems, California, USA) and a commercial western blot assay (Biorad Laboratories, Seattle, Washington, USA). A western blot was considered negative if no virus-specific bands were detected and positive if there was reactivity to at least one glycoprotein (gp41–45, gp120, gp160) and at least three other virus-specific bands. Serum samples were tested for p24 antigen by a commercial assay (Abbott, Chicago, Illinois, USA). A sample was considered positive if the optical density of the sample was greater than that of the control serum provided. Serum β_2-microglobulin concentrations were measured by a quantitative competitive enzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). For the blood donor, β_2-microglobulin could only be measured in samples from the most recent seven visits.

Virus was isolated from PBMC obtained by Ficoll/Hypaque separation from heparinised blood. PBMC (3×10^6/ml) were mixed with an equal number of phytohaemagglutinin-stimulated PBMC (3 μg/ml) from HIV-1-seronegative donors.[9] The cultures were maintained in RPMI 1640 with fetal calf serum (10% by volume) and interleukin-2 (5% by volume; Boehringer-Mannheim, Germany) and the release of p24 core antigen into the supernatants was monitored every 3 days by a standard assay (Genetic Systems). To select CD4 cells by panning,[10] freshly isolated PBMC were first depleted of macrophages by adhesion to tissue culture plastic for 2 h. The non-adherent cells (1.5×10^7/ml) were then incubated with anti-CD4 (10 μg/ml) for 40 min at 4°C. After two washes with cold phosphate-buffered saline containing 1% fetal calf serum, the cells were incubated at 4°C for 2 h on a plate that had been precoated with goat antibody to mouse immunoglobulin and blocked with buffer plus fetal calf serum. All non-adherent cells were removed by five washes with cold buffer. Phytohaemagglutinin-stimulated PBMC from seronegative donors (3×10^6/ml) were added and the plate was incubated overnight at 37°C in 5% carbon dioxide. Cultures were monitored for p24 antigen. The ability of isolated virus strains to induce syncytia was assessed by co-culture with CD4+SUPT1 cells.[11,12] All statistical analyses were done by Fisher's exact test.

Results

We identified 118 cases of transfusion-acquired HIV infection in NSW. By June 30, 1992, AIDS had developed in 68 (58%, 95% confidence interval [CI] 49–67%).

Of the 6 subjects with good outcome infected by a common donor, 2 were identified through tracing of blood units from the known HIV-seropositive donor, 1 was identified through antibody testing as part of screening for clinical illness, and 3 presented for HIV antibody testing because they were worried that blood transfusions in the early 1980s might have put them at risk of HIV infection. There was no significant difference in the proportion of subjects identified as a result of blood tracing between this group (2/6 subjects) and the remaining subjects (30/112; $p = 0.661$).

The blood donor who infected the 6 subjects was a homosexual man with no other risk factors for HIV-1 infection who was 21–23 years old when the implicated blood samples were donated. Between January, 1977, and July, 1984, the subject donated blood 26 times. We could not find out the total number of people who received his blood or blood products. However, he was not implicated in any of the other 112 cases of confirmed transfusion-acquired HIV-1 infection in New South Wales and he had not donated blood elsewhere in Australia.

In December, 1984, the donor was recruited into the Sydney AIDS Prospective Study,[1,13] and he attended follow-up every 6 months between January, 1985, and October, 1991. Information on sexual behaviour was obtained through self-administered questionnaires at each visit. The subject had engaged in receptive anal intercourse only once (in November, 1984). He had engaged in unprotected insertive anal intercourse many times and with many partners in the 6 years up to November, 1984, and on three occasions between November, 1984, and November, 1991.

The donor has had no signs or symptoms of HIV-1 disease at any follow-up examination. His laboratory data are given in table I. He was tested for p24 antigenaemia at all

TABLE I—LABORATORY DATA FOR DONOR, 1984–91

Time (yr) since first recipient infected	Lymphocyte count (× 10⁹/l)	CD4 cells		CD8 cells		CD4/CD8	β_2-microglobulin (mg/l)
		% of T cells	No (× 10⁹/l)	% of T cells	No (× 10⁹/l)		
3·0	1·3	33	0·43	39	0·51	0·85	1·22
3·5	1·8	36	0·65	44	0·80	0·82	ND
4·0	2·2	21	0·46	32	0·70	0·66	ND
4·5	1·6	36	0·56	29	0·45	1·24	ND
5·0	1·8	34	0·61	24	0·43	1·42	ND
5·5	1·6	30	0·48	36	0·58	0·83	ND
6·0	2·0	27	0·54	35	0·70	0·77	ND
6·6	1·5	29	0·44	42	0·63	0·69	ND
7·1	1·8	32	0·58	38	0·68	0·84	ND
7·5	2·3	31	0·71	39	0·90	0·80	1·21
8·0	2·3	26	0·60	40	0·92	0·65	2·51
8·7	2·7	26	0·69	35	0·93	0·74	1·82
9·0	2·4	23	0·55	49	1·18	0·47	1·61
9·3	3·3	22	0·73	49	1·62	0·45	1·27
9·8	2·2	24	0·53	46	1·01	0·52	ND
10·1	2·4	28	0·67	44	1·06	0·64	1·7
Normal range	1·4–2·8	34–54	0·50–1·40	12–28	0·20–0·60	1·4–3·7	< 2·2

ND = not done.

TABLE II—CHARACTERISTICS OF INFECTED RECIPIENTS AT TIME OF TRANSFUSION

Recipient	Sex	Age (yr)	Date of transfusion	Indication for transfusion	Blood product*
A	M	71	August, 1983	Surgery (colectomy)	Red cells
B	F	57	May, 1983	Surgery (acoustic neuroma)	Red cells
C	M	45	January, 1982	Surgery (CABG)	Whole blood
D	M	56	July, 1984	Acute haematemesis	Red cells
E	F	30	June, 1984	Post-partum haemorrhage	Red cells
F	F	18	December, 1982	SLE—renal failure	Platelets

CABG = coronary artery bypass graft; SLE = systemic lupus erythematosus.
*Each subject received 1 unit of blood or blood product.

follow-up visits but was always negative. He was first tested for antibodies to HIV-1 in December, 1984, and he was seropositive at that time. He has never received antiretroviral therapy, nor any prophylactic treatment for *Pneumocystis carinii* pneumonia.

We identified 7 subjects who had received blood or blood products from this donor. A 10-year-old girl received a triple-washed red-cell preparation in March, 1984. She was HIV-1 seronegative when tested 3 years later and has not been retested since then. Her case has been reported previously;[14] there have been other reports of non-transmission of HIV-1 by washed preparations.[15]

Recipients A–F were infected by blood products from the donor between January, 1982, and July, 1984 (table II). HIV-1 infection was first diagnosed in the recipients 2·9–6·5 years after infection. Recipients A–E reported no other risk factor for HIV-1 infection.

Recipients A–E have had no signs or symptoms of HIV-1 disease during follow-up of 6·8 to 10·1 years since transfusion. Recipient F developed *P carinii* pneumonia and died 4·3 years after infection. Since she was not typical of the group and had complicating factors, her case is given in greater detail below. No recipient has been given antiretroviral therapy or prophylaxis against *P carinii* pneumonia.

Each of the 5 living recipients has maintained normal CD4 cell counts (table III), which have not changed substantially in any subject, has remained persistently free of p24 antigenaemia, and has had serum β_2-microglobulin concentrations within the normal laboratory range. Recipient A has had a slight increase in serum β_2-microglobulin with time, but the values are still well within the normal range. Recipient A was the only subject (of the 5 living recipients and the donor) who had a positive virus culture. Standard virus isolation from PBMC and virus isolation after CD4 selection[16] was attempted. The only positive virus cultures were obtained from recipient A in December, 1990 (7·3 years after infection), by CD4 selection from PBMC and in December, 1991 (8·3 years after infection), by culturing PBMC and by CD4 selection. These isolates, HIV-JR1 and HIV-JR2, did not induce syncytia in the SUPT1 co-culture assay. Although both productively infected primary peripheral blood macrophages, productive infection of the HUT-78 or SUPT1 cell lines could not be established.

To find out whether the good outcome among these subjects was statistically better than might generally be expected, we compared this group with 101 other subjects who acquired HIV-1 infection by transfusion in New South Wales and for whom data from 7 years of follow-up were available. The 6 subjects with the common donor were significantly less likely to progress to AIDS or a CD4 count below $0·50 \times 10^9$/l than were the remaining subjects (1/6 *vs* 94/101; $p < 0·0001$; Fisher's exact test).

TABLE III—LABORATORY DATA FOR LIVING INFECTED RECIPIENTS

Time since transfusion (yr)	Lymphocyte count ($\times 10^9$/l)	CD4 cells		CD8 cells		CD4/CD8	β_2-microglobulin (mg/l)
		% of T cells	No ($\times 10^9$/l)	% of T cells	No ($\times 10^9$/l)		
Recipient A							
3·8	2·50	21	0·53	15	0·38	1·40	1·84
4·2	3·00	31	0·93	18	0·54	1·72	2·79
4·4	2·60	20	0·52	8	0·21	2·50	2·65
4·7	2·60	29	0·75	12	0·31	2·42	ND
4·9	2·70	33	0·89	34	0·92	0·97	ND
5·3	2·50	32	0·80	38	0·95	0·84	2·00
5·9	3·00	36	1·08	33	0·99	1·10	ND
6·2	1·61	28	0·46	25	0·41	1·12	3·02
6·4	3·20	27	0·86	36	1·15	0·75	1·64
6·6	2·61	30	0·79	40	1·05	0·75	ND
7·0	2·67	30	0·80	39	1·04	0·77	1·80
7·3	2·55	33	0·84	38	0·97	0·87	3·10
7·6	2·96	22	0·65	46	1·36	0·47	3·60
8·3	2·60	24	0·62	28	0·73	0·86	3·80
Recipient B							
4·6	2·50	38	0·95	21	0·53	1·81	ND
4·9	2·40	45	1·08	24	0·58	1·88	ND
5·2	2·10	38	0·80	36	0·76	1·06	1·60
7·6	3·10	40	1·24	29	0·90	1·38	2·10
8·6	2·80	42	1·18	27	0·76	1·56	2·50
Recipient C							
6·5	2·80	ND	ND	ND	ND	ND	1·62
6·6	2·95	45	0·94	18	0·38	2·50	1·01
7·1	ND	46	ND	29	ND	1·59	0·91
7·6	2·40	50	1·20	26	0·62	1·92	1·03
8·2	2·20	48	1·06	25	0·55	1·92	1·15
8·7	2·10	49	1·03	26	0·55	1·88	1·20
9·1	2·60	46	1·20	25	0·65	1·84	1·34
10·1	2·20	47	1·03	30	0·66	1·57	
Recipient D							
2·9	5·40	27	1·46	34	1·83	0·79	ND
3·2	6·84	26	1·78	15	1·03	1·73	1·92
3·6	6·20	27	1·67	24	1·49	1·13	1·67
3·9	6·33	30	1·90	39	2·47	0·77	1·22
4·5	5·60	29	1·62	41	2·30	0·71	1·32
5·1	5·90	25	1·47	36	2·12	0·69	1·47
5·6	4·65	32	1·49	41	1·91	0·78	ND
6·6	5·30	33	1·75	35	1·85	0·91	1·70
7·1	5·50	31	1·71	34	1·87	0·91	1·58
7·2	7·20	29	2·09	42	3·02	0·69	2·03
7·4	5·50	30	1·65	38	2·09	0·79	1·82
Recipient E							
3·0	1·60	44	0·70	24	0·38	1·83	ND
3·4	1·90	53	1·01	19	0·36	2·79	1·27
4·1	2·40	42	1·01	18	0·43	2·33	1·00
6·2	2·00	48	0·96	24	0·48	2·00	1·78
6·8	2·00	49	0·98	20	0·40	2·45	1·33

ND = not done.
Recipient D underwent splenectomy in August, 1984, 1 mo after HIV infection.

Case-report: Recipient F

Recipient F was an 18-year-old woman who presented in August, 1982, with fever, general myalgia, cutaneous ulceration, and alopecia. Laboratory investigations confirmed the clinical diagnosis of systemic lupus erythematosus (SLE). She responded well to treatment with prednisolone and was discharged 1 month later on maintenance prednisolone. In December, 1982 (after 2 months' non-compliance with medication), she presented with severe shortness of breath and was admitted to hospital. She had had massive pulmonary haemorrhage and within a week of admission she had cardiac arrest. She also had salmonella bacteraemia, extensive herpes simplex virus lesions, staphylococcal pneumonitis, acute respiratory distress syndrome, and renal failure. During the hospital stay she received 98 units of whole blood, 1 of which was from the donor described above. Treatment with

prednisolone and cyclophosphamide was instituted on the day the implicated unit of whole blood was transfused. The patient was discharged 1 month later on maintenance prednisolone.

During the next few years the subject continued treatment with prednisolone for active SLE. In March, 1987, she was admitted to hospital with renal failure, staphylococcal septicaemia, and severe shortness of breath. A chest radiograph showed a bilateral diffuse infiltrate and a silver stain of sputum was positive for *Pneumocystis carinii*. She was positive for antibodies to HIV. T-cell subsets were not counted. She was treated with co-trimoxazole and showed improvement, but died of cardiac, respiratory, and renal complications 2 weeks later.

Discussion

This is the first report of a single common HIV-1-infected donor and a group of transfusion recipients who have all remained symptom-free with normal CD4 cell counts and β_2-microglobulin concentrations and without p24 antigenaemia. Although there have been other reports of long-term HIV-1 infection and normal CD4 counts,[2,8] the lack of a common source of infection precludes investigation of the contributions of host and viral factors to disease progression.

The 6 subjects of our report have had unusually good courses of infection. They were significantly less likely to progress to AIDS or CD4 cell immunodeficiency than were other subjects infected through blood transfusion in New South Wales from whom we had data. There is increasing evidence that the nature of HIV-1 isolates has an important role in determining clinical outcome. Typically, HIV-1 isolates obtained from people with AIDS are virulent and have high replication rates, often with syncytium-inducing capacity and tropism for both T-cell lines and promonocytic cell lines.[12-17] The virulence of the infecting strain of HIV-1 may increase with time and this suggestion has been correlated with the development of severe HIV-1 disease.[12] Our findings suggest that the subjects were infected with a non-pathogenic strain of HIV-1. With conventional virological culture methods we could not isolate HIV-1 from any of our subjects. Although this is not an uncommon finding in people with long-term symptomless infection, there is generally an increase in virus yield after removal of CD8 cells by panning with selective monoclonal antibodies or when the CD4 cells are cultured separately.[16-18] We were able to isolate HIV-1 from the PBMC of 1 subject after CD4 cell selection (and a year later isolated virus from his PBMC without CD4 cell selection). This subject was not the last recipient to be infected, which suggests that the ability to isolate virus was not related to a change in virulence in the donor with time. He was the oldest recipient, and it is possible that involution of the immune system with age made isolation easier. However, the other 4 living recipients remained culture negative. At this stage, we cannot say whether our inability to isolate virus from those subjects indicates viral clearance or a very low number of circulating HIV-1-infected cells. The two viral isolates obtained (HIV-JR1 and HIV-JR2) were non-syncytium-inducing variants and had a restricted host range.

The wide age range of our 6 subjects, the even sex distribution, and the different indications for transfusion reflect a group with no obvious common host factors and therefore argue against the simultaneous occurrence of an effective host immune response among the donor and the recipients. The only recipient who did not have long-term symptomless infection was receiving immunosuppressive agents at the time of HIV-1 infection and she continued to receive these drugs intermittently until she died. Immunosuppressive treatment at the time of primary HIV-1 infection is associated with a poor prognosis.[19-22] Virulent HIV-1 variants may be selectively cleared around the time of seroconversion by the immune system because of higher expression and/or higher immunogenicity.[23] By contrast, low-replicating, non-syncytium-inducing clones can apparently bypass the host immune response and establish persistent infection. The administration of immunosuppressive drugs during primary HIV-1 infection may impair the host immune ability to clear virulent strains of HIV-1 and may allow early expression of these variants. *P carinii* pneumonia has been described in patients with SLE even when only moderate doses of immunosuppressive agents were being used.[24] The contributions of immunosuppression due to active SLE, corticosteroid therapy, or HIV-1 infection to the development of *P carinii* pneumonia in this recipient cannot be estimated.

The 6 subjects for whom follow-up T-cell subset counting has been done have all maintained CD4 cell counts within the normal laboratory range and none has shown any change with time. Recipient A (from whom we were able to isolate HIV-1) had the lowest CD4 count and the highest serum β_2-microglobulin concentrations; he was 1 of 2 subjects whose CD4/CD8 ratio inverted during follow-up (although he remains clinically well). This subject has repeatedly denied any other risk factor for HIV-1 infection and we believe he has not been infected with a strain of HIV-1 in addition to that transmitted from our donor. Previous studies have shown that CD8 cells can inhibit replication of HIV-1, possibly through the production of a lymphokine.[11,25] We have reported a rise in CD8 cells during the first few weeks of primary HIV-1 infection that is associated with clearance of serum p24 antigen.[26] Lifson et al[8] showed that HIV-infected subjects who did not progress had higher CD8 cell counts than a group of subjects who progressed to AIDS. Although the role of CD8 cells in controlling HIV infection is not clear, our findings suggest that factors other than HIV-1 infection itself elicit a CD8 response. It is possible that active viral replication is required.

Serum concentrations of p24 antigen and β_2-microglobulin generally increase with the severity of HIV-1 disease.[27,28] In Lifson and colleagues' study[8] none of their long-term symptom-free subjects had detectable serum p24 antigen. All our subjects were also persistently antigen negative and all had β_2-microglobulin within the normal range.

Few other reports have addressed long-term symptomless HIV infection. Among 24 homosexual men with HIV-1 infection for longer than 5 years, the CD4 cell count remained above $0{\cdot}40 \times 10^9$/l;[8] those who did not progress to AIDS had higher serum β_2-microglobulin concentrations than did seronegative controls, which suggests that there was some immune system activation. In comparison with men who had AIDS, non-progressors had a stronger antibody response to six HIV-1-related proteins but did not differ significantly in neutralising antibody or antibody-dependent cellular cytotoxic activity. Ward et al[7] found that recipients infected by donors in whom AIDS developed within 29 months of donation were more likely to have AIDS 4 years later than were those whose infecting

donors developed AIDS more than 29 months after donation. A self-reported history of sexual intercourse with someone with AIDS is a risk factor for accelerated development of severe HIV-1 disease.[29] Although such reports suggest that virulent strains may be transmitted, ours is the first report to suggest that non-virulent strains continue to be non-virulent in a new host.

Whether our subjects will ultimately develop severe HIV-1 disease or signs of immunodeficiency may not be known for several years. However, we believe that the identification of this group of subjects should stimulate the search for other similar groups. They can provide information on the immunopathogenesis of HIV-1 disease and on the nature of effective host immune responses that will be vital for the development of effective vaccine strategies.

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From The Lancet

Breakfast with the Borgias

Poisoners have flourished since man first discovered that some changes in the internal environment are more than the living organism will bear. Nor has the law hesitated to make use of this peculiarity of protoplasm; Dr G. Roche Lynch, addressing the Medico-Legal Society as its retiring president on July 16, quoted an early Egyptian papyrus where "the penalty of the peach" showed prussic acid to be the recognised means of liquidating malefactors. The Greeks, of course, used hemlock; and he reminds us that the word "toxic" derived from *τόξον*—the bow which discharged a poisoned arrow. In medieval England poisoning was so repulsive to the legal mind, however, that the statutory punishment for the poisoner was to be boiled in oil—a highly unfair arrangement since the tests for deciding whether death was due to poisoning were far from satisfactory. Thus it was believed that a poisoned body—and especially the heart—resisted fire more successfully than one in which death had been natural. . . . Statistics of homicidal poisoning—for reasons beyond the control of the Registrar-General—are inaccurate. Probably only a proportion of murders by poison come to light. Dr Roche Lynch recalled that out of a series of 24 murders by poison, 8 were only discovered by exhumation; and that many poisoners have killed more than one person. The more cautious ones probably escape detection, but those who are apprehended show a strongly conservative spirit in their choice of drug. Arsenic has always been a favourite because of its small bulk, but large doses are often necessary. Opium can still be obtained in spite of legal safeguards, and Nurse Waddingham saved up a quantity which had been prescribed for dying patients under her care. . . . Weed killer and industrial poisons like cyanide can be acquired fairly easily but attempts by a layman to buy an unusual poison are readily traced, and this limits the murder's choice.

(Aug 1, 1942)